NOTES ON UROGLENA AMERICANA Calk.

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(WITH PLATE X)

In November 1895, at the suggestion of Dr. Farlow, I obtained specimens of the peculiar organism described by Calkins (1) as *Uroglena Americana*, and attempted to make some observations concerning it. The genus Uroglena, established by Ehrenberg (2) in 1833, referred to by Bütschli (3) and Stein (4), and considered at some length by Kent (5), has been observed in the public water supplies of Massachusetts and Connecticut with more or less regularity since 1889. In addition to the original species, Calkins (6) has found two others which he describes as *U. radiata* and *U. Americana*. Thus far I have been able to examine only *U. Americana*, and the following observations have been made upon that species.

While neither *U. volvox* nor *U. radiata* have been reported as causing any perceptible change in the water, *U. Americana* produces a very disagreeable odor, and a decided fishy oily taste. In the pond at Norwood, Mass., where all the material was obtained, the water was almost unfit for use, and caused great inconvenience. Calkins (7) seems to have successfully shown that this peculiarity of the species is due to the presence of numerous oil globules in the individual cells, and that contamination takes place through the liberation of this oil rather than from decay. The individual cells, as well as the colonies, are extremely delicate and the slightest disturbance is apt to break them up. While the water in the pond at Norwood was not noticeably disagreeable, the process of pumping it through several miles of pipe into a reservoir was sufficient to completely disintegrate the cells, and thus the reservoir water became polluted through the mechanical breaking of the colonies and cells, and consequent liberation of the oil.

THE CŒNOBIUM.

The colony of *U. Americana* presents a somewhat similar appearance to that of *Volvox globator* L. That is, it consists of a more or less spherical sac of transparent jelly, in the periphery of which are numerous green cells provided with cilia which cause the organism to rotate slowly through the water. There the resemblance ceases, and in no way can the two be said to have a generic relation.

In size and shape the Uroglena cœnobium varies greatly. While the general outline may be spherical, it is frequently found with protuberances and irregularities. All stages, from that of a perfect globe to a long cylinder with closed ends, have been observed, and many modifications of these extreme forms are apt to occur. The size varies as much as the shape. From the first early stages, consisting of but a few cells and measuring 30–40μ in diameter, we may have all gradations up to the somewhat unusual size of 525μ containing hundreds of individual cells. In the latter case the colony had been kept for some time under most favorable conditions, and probably represents the maximum growth.

The individual cells are irregularly placed, and from 10–20μ apart. There are no connecting canals as in Volvox. In regard to the structure of the interior of the colony of the original species (*U. volvox* Ehren.) there has been quite a difference of opinion. Ehrenberg (6) held that the contents were fluid, and the individual cells were drawn out into "tails," all these "tails" being united at a common point in the center of the cœnobium. Neither Stein nor Bütschli observed anything of this kind, and considered it very improbable, Stein even maintaining that the colony was a homogeneous mass of jelly from center to circumference. Kent (5) confirmed the observations of Ehrenberg in regard to the appendage of the individual cells, and suggested that they might be contractile. Zacharias (7), in a recent article, brings forward the view that *Uroglena volvox* does possess an internal network of threads or "tubes," but he further maintains that the prolongation from each individual cell is not in direct

communication with the center of the colony. Instead, he finds that the interior of the cœnobium is filled with a system of dichotomously branched threads, which radiate in all directions from a common center, and near the periphery unite as a single filament with the base of the individual cell.

Coming to the condition of things in *U. Americana*, there can be no doubt that such an arrangement of threads does not exist. In fact, no prolongation of the individual cell is found in any form. Careful staining with alum hæmatoxylin (the method used by Zacharias) failed to reveal the slightest trace of any connection of the cells with the interior of the colony, and various other methods were tried with the same negative result. In addition to the test of the stains, the manner in which the colony breaks up would indicate that there is no "central binding structure," for *U. Americana* is characterized by the extreme delicacy of its colonies, and while other species will stand a reasonable amount of manipulation, this form begins to separate upon the slightest change of condition, and certainly does not assume the definite arrangement of *U. radiata*, for example. Finally, the fact that numerous protozoa swim here and there in the cœnobium without obstruction, and colonies half the size of the enclosing one are found revolving freely within, would seem to show conclusively that no network of threads, as described by Zacharias in *U. volvox*, could exist in this species.

THE INDIVIDUAL CELL.

The individual cells, which are placed in the periphery of the jelly like globe, vary slightly in size, ranging from 7–11μ in diameter. The great majority are spherical, but occasionally the end towards the center of the colony will be slightly tapering. In no case, however, do they approach the long drawn out appearance of those cells figured by Ehrenberg and Zacharias, and *U. Americana* is most definitely defined by the spherical outline of its individual cells.

Each cell is provided with two cilia of unequal length, the longer sometimes reaching 20μ, the shorter seldom more than

4μ. At the base of the cilia is found an elliptical or oblong red spot, and a well defined nucleus is located near the center of the cell. One or two vacuoles of a non-contractile character are present, and numerous oil globules distribute themselves throughout the individual. There is but one chromatophore, which is yellowish green in color. This usually clings close to one side of the cell, or may occupy the end towards the circumference of the colony. The base of the cell is sometimes filled with oil globules, but is generally hyaline. Previous to the observations of Zacharias two chromatophores were reported as being present, but he demonstrated the fact that while in *U. volvox* the chromatophore frequently assumed a spiral arrangement, which made it appear divided, there was in reality never but the one color body. In *U. Americana* there is no spiral arrangement noted, and little or no difficulty is experienced in making out the single chromatophore. When the cells are ready to divide, however, there are two chromatophores present, and this may have caused the error in former observations.

The division of the individual cell takes place in the following manner. A single cell begins to turn so that the cilia are in a plane with the tangent of the sphere, instead of at right angles. The chromatophore divides and occupies opposite sides of the cell and a new red spot makes its appearance somewhat away from the old one, but not necessarily in the place where the new cilia are to originate. At the point directly opposite that at which the old cilia are located a new pair of cilia are formed, and we then have a somewhat larger cell with two chromatophores, two red spots, and two sets of cilia at opposite sides. All of this takes place before the cell begins to elongate or divide in any perceptible manner. After the new pair of cilia is completed the cell begins to lengthen in the direction of its cilia, and in a short time an oblong cell, nearly three times as long as wide, with a pair of cilia at each end, is formed. It is now that actual division begins to take place, and it only requires a few minutes to complete the operation. Halfway between the two pairs of cilia a constriction appears, and while

it extends entirely around the cell the depression is always deepest at the side nearest the periphery of the colony. Thus the pressure is greatest from that side, and consequently the halves of the dividing cell are gradually turned at right angles to their former position, and at the time when complete division takes place present their normal appearance, viz., with cilia at right angles to the tangent of the cœnobium. The red spot, which may have been in almost any part of the cell at first, takes its place at the base of the cilia before the final separation occurs. A reference to the figures will explain better than any description just how this division takes place.

THE RESTING STAGE.

Under certain conditions it is possible for an individual cell to lose its cilia and, forming a thick gelatinous wall, go into a resting stage. When this occurs the chromatophore breaks up and the chlorophyll is distributed throughout the entire cell, the red spot wholly disappearing. After a time the contents of this encysted cell divides and forms two elliptical bodies, and these in turn dividing we have four elliptical cells within the original cyst wall. Each daughter cell is provided with a red spot and a pair of cilia before the wall is ruptured, and so is ready to begin the process of division and formation of a new cœnobium as soon as liberated.

When the cells are first set free the chromatophore does not occupy the definite position that it does later, but is distributed equally throughout the contents of the cell, and is of a brighter green color. Oil globules are very abundant at this time, and give the cells a decided granular appearance. In a very few instances a cyst was observed that had divided into eight daughter cells. This was mentioned by Kent (5), but does not seem to be the general rule, and certainly is not necessary.

TAXONOMIC POSITION.

From the large number of colonies examined, and the length of time the observations covered, it would seem probable that

enough negative evidence had been secured to justify our considering Uroglena as being among those forms which have no sexual mode of reproduction. It is certain that up to the present time nothing has been observed that can in any way be considered as indicating anything but the simplest methods of multiplication. Kent (5) observed bodies which he designated as "microspores" and "macrospores," but that is the most that can be said in regard to the fact. Zacharias (7) calls attention to larger cells in the periphery of the cœnobium, containing two red spots and two chromatophores, which he names "zygote formers." Since he does not describe the process of conjugation, one is led to believe that it had not been observed and, for the present at least, the term zygospore will have to be classed with the microspores and macrospores of Kent. It naturally occurs to one that the so called zygote forming cells of Zacharias were merely ordinary individual cells about ready to begin the process of division.

It would seem, then, since the only known method of reproduction is by simple division, that the taxonomic position of Uroglena, if it is to be regarded as a plant, must be among the multicellular Chrysomonadaceæ of the class Syngeneticæ.

It is so placed by Warming (8), and more recently by E. Lemmermann (9), and while the characters of the genus are hardly in accord with the family Syngeneticæ as defined by Rostafinski, still it would seem that under the generally accepted idea of the Chrysomonadaceæ Uroglena would find a place in that order, together with Syncrypta.

From the foregoing account it will be seen that *U. Americana* varies decidedly from the description of *U. volvox* as given by Ehrenberg, Zacharias, and others. The fact that the European species is found most abundantly during the summer, while here the colder months are more favorable to its growth, may account for some of the minor variations. It seems probable, however, that what has been considered *U. volvox* by previous observers has not always been the same species, and that much of the inability to agree, and the surprise expressed by more recent writers that certain structures had not been seen by former investigators,

is due to this fact. According to Zacharias the cilia of *U. volvox* are more nearly of the same length, there are no vacuoles or oil globules, and the individual cells are elliptical or oblong, being invariably drawn out into a tapering point which is prolonged into a thread. On the other hand, *U. Americana*, as shown, contains no network of threads, and the individual cells are spherical in outline, not being prolonged in any way. The single chromatophore seems to be common to both species. The method of reproduction in *U. volvox* has not been satisfactorily demonstrated and no comparison can be made; however, nothing has been observed thus far, that would make it improbable that the peculiar method of multiplication as described above for *U. Americana* does not exist in the original species.

For convenience I append a somewhat modified description of this species as given by Calkins:

UROGLENA AMERICANA Calkins, 23d Ann. Rep. Mass. State Board of Health, 1891.—*Cœnobium:* irregularly spherical, varying greatly in shape and size, averaging 200–300 μ; no peripheral canals or internal network of threads; revolves slowly through the water by means of cilia of individual cells. *Individual cells:* spherical or occasionally slightly elliptical, never prolonged into an appendage at end towards center of colony; two cilia of unequal length, 15–20 μ and 2–4 μ respectively, the longer with decided undulatory motion; red spot at base of cilia and a single chromatophore, of a yellowish green color, usually occupying one side of the cell and clinging close to the wall; nucleus, non-contractile vacuoles, and numerous oil globules present.

Water supplies of Massachusetts and Connecticut. September-June.[1]

I desire to acknowledge my indebtedness to Professor Farlow for his interest and advice, also for the loan of valuable and necessary literature on the subject.

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[1] Since the above went to press, Uroglena has been reported from Indiana by Mr. S. Burrage of Purdue University, and probably will be found to be widely distributed.

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EXPLANATION OF PLATE X.

Uroglena Americana Calk.

FIG. 1. General appearance of colony, × 333.

FIG. 2. Individual cell, × 2000.

FIG. 3. Individual cell with two chromatophores, two red spots and two sets of cilia, ready to elongate, × 2000.

FIGS. 4–7. Successive stages in the division, showing manner in which cell turns from horizontal to vertical position, × 2000.

FIGS. 8–11. Encysted forms, × 1000.

FIG. 8. Before division.

FIGS. 9–11. Various stages in formation of daughter cells.

All drawn from nature, and as far as possible sketched with an Abbé camera.